

CLAIMS

WHAT IS CLAIMED IS:

1. A recombinant respiratory syncytial virus (RSV) having an attenuated phenotype and comprising a phosphoprotein (P), which phosphoprotein comprises at least one artificially mutated amino acid residue.
2. The recombinant RSV of claim 1, wherein the phosphoprotein comprises at least one substituted amino acid residue.
3. The recombinant RSV of claim 1, wherein the phosphoprotein comprises a deletion of at least one amino acid residue.
4. The recombinant RSV of claim 1, wherein the phosphoprotein comprises at least one mutated amino acid residue at a position selected from the group consisting of position 172, position 174, position 175 and position 176.
5. The recombinant RSV of claim 4, wherein the phosphoprotein comprises at least one substituted amino acid residue at a position selected from the group consisting of position 172, position 174, position 175 and position 176.
6. The recombinant RSV of claim 5, wherein the phosphoprotein comprises a glycine to serine substitution at position 172 (G172S), or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.
7. The recombinant RSV of claim 5, wherein the phosphoprotein comprises an arginine to alanine substitution at position 174 (R174A), or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.
8. The recombinant RSV of claim 5, wherein the phosphoprotein comprises a glutamic acid to alanine substitution at position 175 (E175A), or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.
9. The recombinant RSV of claim 5, wherein the phosphoprotein comprises a glutamic acid to glycine substitution at position 176 (E176G), a glutamic acid to alanine substitution at position 176 (E176A), a glutamic acid to aspartic acid substitution at position 176 (E176D), a glutamic acid to cysteine substitution at position 176 (E176C) or

a glutamic acid to serine substitution at position 176 (E176S), or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.

10. The recombinant RSV of claim **5**, wherein the phosphoprotein comprises substituted amino acid residues at positions 172 and 176.

11. The recombinant RSV of claim **1**, wherein the phosphoprotein comprises a plurality of substituted amino acid residues, which residues are selected from residues 172-176.

12. The recombinant RSV of claim **11**, wherein the phosphoprotein comprises an arginine to alanine substitution at position 174 (R174A), a glutamic acid to alanine substitution at position 175 (E175A), and a glutamic acid to alanine substitution at position 176 (E176A), or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.

13. The recombinant RSV of claim **1**, wherein the phosphoprotein comprises a deletion of a plurality of amino acid residues selected from residues 172-176.

14. The recombinant RSV of claim **13**, wherein the phosphoprotein comprises a deletion of amino acid residues 172-176, or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.

15. The recombinant RSV of claim **13**, wherein the phosphoprotein comprises a deletion of amino acid residues 161-180, or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.

16. The recombinant RSV of claim **1**, wherein the phosphoprotein comprises a deletion of a plurality of amino acid residues selected from residues 236-241.

17. The recombinant RSV of claim **16**, wherein the phosphoprotein comprises a deletion of amino acid residues 236-241, or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.

18. The recombinant RSV of claim **1**, wherein the at least one mutated amino acid residue eliminates a phosphorylation site.

19. The recombinant RSV of claim **1**, wherein the phosphoprotein comprises at least one substituted amino acid residue, which substituted amino acid residue eliminates a phosphorylation site.

- 20.** The recombinant RSV of claim **19**, wherein the at least one substituted amino acid residue replaces a serine.
- 21.** The recombinant RSV of claim **19**, wherein the at least one substituted amino acid residue replaces a serine at one or more positions selected from the group consisting of positions 116, 117, 119, 232 and 237.
- 22.** The recombinant RSV of claim **21**, wherein the phosphoprotein comprises amino acid substitution S116D, amino acid substitution S116A or amino acid substitution S116L, or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.
- 23.** The recombinant RSV of claim **21**, wherein the phosphoprotein comprises amino acid substitution S117D, amino acid substitution S117A, or amino acid substitution S117R, or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.
- 24.** The recombinant RSV of claim **21**, wherein the phosphoprotein comprises amino acid substitution S119D, amino acid substitution S119A, or amino acid substitution S119L, or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.
- 25.** The recombinant RSV of claim **21**, wherein the phosphoprotein comprises amino acid substitution S232A or amino acid substitution S232D, or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.
- 26.** The recombinant RSV of claim **21**, wherein the phosphoprotein comprises amino acid substitution S237A or amino acid substitution S237D, or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.
- 27.** The recombinant RSV of claim **21**, wherein substituted amino acid residues replace serines at positions 117 and 119.
- 28.** The recombinant RSV of claim **27**, wherein the phosphoprotein comprises an amino acid substitution selected from the group consisting of S117A, S117D and S117R and an amino acid substitution selected from the group consisting of S119A, S119D and S119L, or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.

- 29.** The recombinant RSV of claim **21**, wherein substituted amino acid residues replace serines at positions 116, 117 and 119.
- 30.** The recombinant RSV of claim **29**, wherein the substituted amino acid residue at position 116 is selected from the group consisting of alanine (S116A), aspartic acid (S116D) and leucine (S116L), or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.
- 31.** The recombinant RSV of claim **29**, wherein the substituted amino acid residue at position 117 is selected from the group consisting of alanine (S117A), aspartic acid (S117D) and arginine (S117R), or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.
- 32.** The recombinant RSV of claim **29**, wherein the substituted amino acid residue at position 119 is selected from the group consisting of alanine (S119A), aspartic acid (S119D) and leucine (S119L), or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.
- 33.** The recombinant RSV of claim **29**, wherein the phosphoprotein comprises an amino acid substitution selected from the group consisting of S116L, S116A, and S116D; an amino acid substitution selected from the group consisting of S117R, S117A, and S117D; and an amino acid substitution selected from the group consisting of S119L, S119A, and S119D, or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.
- 34.** The recombinant RSV of claim **21**, wherein substituted amino acid residues replace serines at positions 232 and 237.
- 35.** The recombinant RSV of claim **34**, wherein the substituted amino acid residue at position 232 is selected from the group consisting of alanine (S232A) and aspartic acid (S232D), or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.
- 36.** The recombinant RSV of claim **34**, wherein the substituted amino acid residue at position 237 is selected from the group consisting of alanine (S237A) and aspartic acid (S237D), or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.

- 37.** The recombinant RSV of claim **34**, wherein the phosphoprotein comprises an amino acid substitution selected from the group consisting of S232A and S232D and an amino acid substitution selected from the group consisting of S237A and S237D, or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.
- 38.** The recombinant RSV of claim **21**, wherein substituted amino acid residues replace serines at positions 116, 117, 119, 232 and 237.
- 39.** The recombinant RSV of claim **38**, wherein the substituted amino acid residue at position 116 is selected from the group consisting of leucine (S116L), alanine (S116A) and aspartic acid (S116D), or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.
- 40.** The recombinant RSV of claim **38**, wherein the substituted amino acid residue at position 117 is selected from the group consisting of arginine (S117R), alanine (S117A) and aspartic acid (S117D), or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.
- 41.** The recombinant RSV of claim **38**, wherein the substituted amino acid residue at position 119 is selected from the group consisting of leucine (S119L), alanine (S119A) and aspartic acid (S119D), or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.
- 42.** The recombinant RSV of claim **38**, wherein the substituted amino acid residue at position 232 is selected from the group consisting of alanine (S232A) and aspartic acid (S232D), or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.
- 43.** The recombinant RSV of claim **38**, wherein the substituted amino acid residue at position 237 is selected from the group consisting of alanine (S237A) and aspartic acid (S237D), or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein.
- 44.** The recombinant RSV of claim **38**, wherein the phosphoprotein comprises an amino acid substitution selected from the group consisting of S116L, S116A, and S116D; an amino acid substitution selected from the group consisting of S117R, S117A, and S117D; an amino acid substitution selected from the group consisting of S119L, S119A, and

S119D; an amino acid substitution selected from the group consisting of S232A and S232D; and an amino acid substitution selected from the group consisting of S237A and S237D, or wherein the phosphoprotein is an artificial conservative variation of such a phosphoprotein

45. The recombinant RSV of claim 1, wherein the recombinant RSV comprises a human RSV of subgroup A, subgroup B or a chimera thereof.

46. A nucleic acid encoding the recombinant RSV of claim 1.

47. The nucleic acid of claim 46, wherein the nucleic acid is a DNA.

48. The nucleic acid of claim 47, wherein the DNA is a cDNA.

49. The nucleic acid of claim 46, wherein the nucleic acid is an RNA.

50. The nucleic acid of claim 46, wherein the nucleic acid is an RSV genome or antigenome.

51. A vector comprising the nucleic acid of claim 46.

52. The vector of claim 51, comprising a plasmid.

53. The phosphoprotein of claim 1, or an artificial conservative variation thereof.

54. A nucleic acid encoding the phosphoprotein of claim 53.

55. A live attenuated respiratory syncytial virus vaccine comprising an immunologically effective amount of the recombinant RSV of claim 1.

56. The vaccine of claim 55, further comprising a physiologically acceptable carrier.

57. The vaccine of claim 55, further comprising an adjuvant.

58. A method for stimulating the immune system of an individual to produce an immune response against RSV, the method comprising administering to the individual the recombinant RSV of claim 1 in a physiologically acceptable carrier.

59. The method of claim 58, wherein the immune response is a protective immune response.

60. The method of claim 58, wherein the recombinant RSV is administered to the upper respiratory tract of the individual.

- 61.** The method of claim **60**, wherein the recombinant RSV is administered to the nasopharynx.
- 62.** The method of claim **58**, wherein the recombinant RSV is administered by spray, droplet or aerosol.
- 63.** A method of identifying a phosphoprotein or nucleoprotein having altered interaction with another protein, the method comprising:
- providing a plurality of protein variants, each protein variant comprising at least a portion of a first RSV protein, which first RSV protein is selected from the group consisting of an RSV phosphoprotein and an RSV nucleoprotein, and which portion of an RSV protein comprises at least one artificial mutation; and,
 - identifying at least one candidate protein variant having an altered interaction with a second RSV protein or portion thereof.
- 64.** The method of claim **63**, wherein the first RSV protein is an RSV phosphoprotein and the second RSV protein is an RSV nucleoprotein.
- 65.** The method of claim **63**, wherein the first RSV protein is an RSV nucleoprotein and the second RSV protein is an RSV phosphoprotein.
- 66.** The method of claim **63**, wherein identifying at least one candidate protein variant having an altered interaction with a second RSV protein comprises performing an in vivo assay.
- 67.** The method of claim **63**, wherein identifying at least one candidate protein variant having an altered interaction with a second RSV protein comprises performing an in vitro assay.
- 68.** The method of claim **63**, wherein the altered interaction is a decreased interaction.
- 69.** The method of claim **63**, wherein the interaction is altered in a temperature-dependent manner.
- 70.** The method of claim **63**, further comprising determining the nature of the at least one mutation in the portion of the first RSV protein comprising at least one of the candidate protein variants.

- 71.** The method of claim **63**, further comprising producing at least one recombinant RSV, the genome or antigenome of which encodes a phosphoprotein or a nucleoprotein comprising the at least one mutation in the portion of the first RSV protein comprising at least one of the candidate protein variants.
- 72.** The method of claim **71**, further comprising identifying at least one recombinant RSV having a reduced level of replication.
- 73.** The method of claim **72**, wherein replication is reduced at least 10-fold.
- 74.** The method of claim **72**, wherein replication is reduced at least 100-fold.
- 75.** The method of claim **72**, wherein identifying at least one recombinant RSV having a reduced level of replication comprises assessing replication in cultured cells.
- 76.** The method of claim **72**, wherein identifying at least one recombinant RSV having a reduced level of replication comprises assessing replication in an animal.
- 77.** The method of claim **76**, wherein the animal is a rodent or a primate.
- 78.** The method of claim **72**, wherein identifying at least one recombinant RSV having a reduced level of replication comprises assessing replication in a human.
- 79.** A recombinant respiratory syncytial virus (RSV) having an attenuated phenotype and comprising an M2-1 protein, which M2-1 protein comprises at least one artificially mutated amino acid residue at a position selected from the group consisting of position 3, position 12, position 14, position 16, position 17, and position 20.
- 80.** The recombinant RSV of claim **79**, wherein the M2-1 protein comprises at least one substituted amino acid residue at a position selected from the group consisting of position 3, position 12, position 14, position 16, position 17, and position 20.
- 81.** The recombinant RSV of claim **80**, wherein the M2-1 protein comprises an arginine to valine substitution at position 3 (R3V), or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.
- 82.** The recombinant RSV of claim **80**, wherein the M2-1 protein comprises an arginine to glutamine substitution at position 12 (R12Q), or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.

- 83.** The recombinant RSV of claim **80**, wherein the M2-1 protein comprises a histidine to phenylalanine substitution at position 14 (H14F), or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.
- 84.** The recombinant RSV of claim **80**, wherein the M2-1 protein comprises a leucine to serine substitution at position 16 (L16S), or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.
- 85.** The recombinant RSV of claim **80**, wherein the M2-1 protein comprises an asparagine to arginine substitution at position 17 (N17R), or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.
- 86.** The recombinant RSV of claim **80**, wherein the M2-1 protein comprises an arginine to asparagine substitution at position 20 (R20N), or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.
- 87.** The recombinant RSV of claim **80**, wherein the M2-1 protein comprises amino acid substitutions L16S and N17R, or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.
- 88.** The recombinant RSV of claim **80**, wherein the M2-1 protein comprises amino acid substitutions R12Q and H14F, or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.
- 89.** The recombinant RSV of claim **80**, wherein the M2-1 protein comprises amino acid substitutions R12Q and R20N, or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.
- 90.** The recombinant RSV of claim **80**, wherein the M2-1 protein comprises amino acid substitutions H14F and R20N, or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.
- 91.** The recombinant RSV of claim **80**, wherein the M2-1 protein comprises amino acid substitutions R12Q, H14F and R20N, or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.
- 92.** The recombinant RSV of claim **79**, wherein the recombinant RSV comprises a human RSV of subgroup A, subgroup B or a chimera thereof.

- 93.** A nucleic acid encoding the recombinant RSV of claim **79**.
- 94.** The nucleic acid of claim **93**, wherein the nucleic acid is a DNA.
- 95.** The nucleic acid of claim **94**, wherein the DNA is a cDNA.
- 96.** The nucleic acid of claim **93**, wherein the nucleic acid is an RNA.
- 97.** The nucleic acid of claim **93**, wherein the nucleic acid is an RSV genome or antigenome.
- 98.** A vector comprising the nucleic acid of claim **93**.
- 99.** The vector of claim **98**, comprising a plasmid.
- 100.** The M2-1 protein of claim **79**, or an artificial conservative variation thereof.
- 101.** A nucleic acid encoding the M2-1 protein of claim **100**.
- 102.** A live attenuated respiratory syncytial virus vaccine comprising an immunologically effective amount of the recombinant RSV of claim **79**.
- 103.** The vaccine of claim **102**, further comprising a physiologically acceptable carrier.
- 104.** The vaccine of claim **102**, further comprising an adjuvant.
- 105.** A method for stimulating the immune system of an individual to produce an immune response against RSV, the method comprising administering to the individual the recombinant RSV of claim **79** in a physiologically acceptable carrier.
- 106.** The method of claim **105**, wherein the immune response is a protective immune response.
- 107.** The method of claim **105**, wherein the recombinant RSV is administered to the upper respiratory tract of the individual.
- 108.** The method of claim **107**, wherein the recombinant RSV is administered to the nasopharynx.
- 109.** The method of claim **105**, wherein the recombinant RSV is administered by spray, droplet or aerosol.
- 110.** A recombinant respiratory syncytial virus (RSV) having an attenuated phenotype and comprising a chimeric M2-1 protein, which chimeric M2-1 protein comprises a plurality

of residues from an RSV M2-1 protein and a plurality of residues from a pneumonia virus of mice (PVM) M2-1 protein.

111. The recombinant RSV of claim **110**, wherein the chimeric M2-1 protein comprises a plurality of residues from the N-terminal region of the RSV M2-1 protein and a plurality of residues from the C-terminal region of the PVM M2-1 protein.

112. The recombinant RSV of claim **111**, wherein the chimeric M2-1 protein comprises the N-terminal 30 residues of the RSV M2-1 protein and the C-terminal 148 residues of the PVM M2-1 protein, or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.

113. The recombinant RSV of claim **110**, wherein the chimeric M2-1 protein comprises a plurality of residues from the N-terminal region of the PVM M2-1 protein and a plurality of residues from the C-terminal region of the RSV M2-1 protein.

114. The recombinant RSV of claim **113**, wherein the chimeric M2-1 protein comprises the N-terminal 29 residues of the PVM M2-1 protein and the C-terminal 164 residues of the RSV M2-1 protein, or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.

115. The recombinant RSV of claim **114**, wherein the chimeric M2-1 protein further comprises at least one substituted amino acid residue at a position selected from the group consisting of position 3, position 11, position 13, position 15, position 16, position 19 and position 25.

116. The recombinant RSV of claim **115**, wherein the chimeric M2-1 protein comprises a valine to arginine substitution at position 3 (V3R), or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.

117. The recombinant RSV of claim **115**, wherein the chimeric M2-1 protein comprises a glutamine to arginine substitution at position 11 (Q11R), or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.

118. The recombinant RSV of claim **115**, wherein the chimeric M2-1 protein comprises a serine to leucine substitution at position 15 (S15L), or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.

- 119.** The recombinant RSV of claim **115**, wherein the chimeric M2-1 protein comprises an arginine to asparagine substitution at position 16 (R16N), or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.
- 120.** The recombinant RSV of claim **115**, wherein the chimeric M2-1 protein comprises an asparagine to arginine substitution at position 19 (N19R), or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.
- 121.** The recombinant RSV of claim **115**, wherein the chimeric M2-1 protein comprises amino acid substitutions S15L and R16N, or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.
- 122.** The recombinant RSV of claim **115**, wherein the chimeric M2-1 protein comprises amino acid substitutions Q11R and F13H, or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.
- 123.** The recombinant RSV of claim **115**, wherein the chimeric M2-1 protein comprises amino acid substitutions Q11R, F13H, and N19R, or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.
- 124.** The recombinant RSV of claim **115**, wherein the chimeric M2-1 protein comprises amino acid substitutions V3R, S15L and R16N, or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.
- 125.** The recombinant RSV of claim **115**, wherein the chimeric M2-1 protein comprises amino acid substitutions Q11R, S15L and R16N, or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.
- 126.** The recombinant RSV of claim **115**, wherein the chimeric M2-1 protein comprises amino acid substitutions S15L, R16N and N19R, or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.
- 127.** The recombinant RSV of claim **115**, wherein the chimeric M2-1 protein comprises amino acid substitutions Q11R, F13H, S15L and R16N, or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.
- 128.** The recombinant RSV of claim **115**, wherein the chimeric M2-1 protein comprises amino acid substitutions Q11R, F13H, S15L, R16N and N19R, or wherein the M2-1 is an artificial conservative variation of such an M2-1 protein.

- 129.** The recombinant RSV of claim **110**, wherein the recombinant RSV comprises a human RSV of subgroup A, subgroup B or a chimera thereof.
- 130.** A nucleic acid encoding the recombinant RSV of claim **110**.
- 131.** The nucleic acid of claim **130**, wherein the nucleic acid is a DNA.
- 132.** The nucleic acid of claim **131**, wherein the DNA is a cDNA.
- 133.** The nucleic acid of claim **130**, wherein the nucleic acid is an RNA.
- 134.** The nucleic acid of claim **130**, wherein the nucleic acid is an RSV genome or antigenome.
- 135.** A vector comprising the nucleic acid of claim **130**.
- 136.** The vector of claim **135**, comprising a plasmid.
- 137.** The chimeric M2-1 protein of claim **110**, or an artificial conservative variation thereof.
- 138.** A nucleic acid encoding the chimeric M2-1 protein of claim **137**.
- 139.** A live attenuated respiratory syncytial virus vaccine comprising an immunologically effective amount of the recombinant RSV of claim **110**.
- 140.** The vaccine of claim **139**, further comprising a physiologically acceptable carrier.
- 141.** The vaccine of claim **139**, further comprising an adjuvant.
- 142.** A method for stimulating the immune system of an individual to produce an immune response against RSV, the method comprising administering to the individual the recombinant RSV of claim **110** in a physiologically acceptable carrier.
- 143.** The method of claim **142**, wherein the immune response is a protective immune response.
- 144.** The method of claim **142**, wherein the recombinant RSV is administered to the upper respiratory tract of the individual.
- 145.** The method of claim **144**, wherein the recombinant RSV is administered to the nasopharynx.

146. The method of claim **142**, wherein the recombinant RSV is administered by spray, droplet or aerosol.

147. A method of identifying an M2-1 protein having an altered activity, the method comprising:

providing one or more chimeric M2-1 proteins, each of which chimeric M2-1 protein comprises a plurality of residues from an RSV M2-1 protein from a first strain of virus and a plurality of residues from an M2-1 protein from a second strain of virus; and, identifying at least one candidate chimeric M2-1 protein having an altered activity.

148. The method of claim **147**, wherein the first and second strains of virus are different strains of RSV.

149. The method of claim **147**, wherein the first and second strains of virus are different species of virus.

150. The method of claim **149**, wherein at least one of the chimeric M2-1 proteins comprises a plurality of residues from an RSV M2-1 protein and a plurality of residues from a pneumonia virus of mice (PVM) M2-1 protein.

151. The method of claim **150**, wherein the chimeric M2-1 protein comprises a plurality of residues from the N-terminal region of the RSV M2-1 protein and a plurality of residues from the C-terminal region of the PVM M2-1 protein.

152. The method of claim **150**, wherein the chimeric M2-1 protein comprises a plurality of residues from the N-terminal region of the PVM M2-1 protein and a plurality of residues from the C-terminal region of the RSV M2-1 protein.

153. The method of claim **147**, wherein identifying at least one candidate chimeric M2-1 protein having an altered activity comprises assaying M2-1-dependent processivity.

154. The method of claim **147**, wherein identifying at least one candidate chimeric M2-1 protein having an altered activity comprises assaying RNA binding by the candidate chimeric M2-1 protein.

155. The method of claim **147**, wherein identifying at least one candidate chimeric M2-1 protein having an altered activity comprises assaying nucleoprotein binding by the candidate chimeric M2-1 protein.

- 156.** The method of claim **147**, further comprising producing at least one recombinant respiratory syncytial virus (RSV), the genome or antigenome of which encodes at least one candidate chimeric M2-1 protein.
- 157.** The method of claim **156**, further comprising identifying at least one recombinant RSV having a reduced level of replication.
- 158.** The method of claim **157**, wherein replication is reduced at least 10-fold.
- 159.** The method of claim **157**, wherein replication is reduced at least 100-fold.
- 160.** The method of claim **157**, wherein identifying at least one recombinant RSV having a reduced level of replication comprises assessing replication in cultured cells.
- 161.** The method of claim **157**, wherein identifying at least one recombinant RSV having a reduced level of replication comprises assessing replication in an animal.
- 162.** The method of claim **161**, wherein the animal is a rodent or a primate.
- 163.** The method of claim **157**, wherein identifying at least one recombinant RSV having a reduced level of replication comprises assessing replication in a human.
- 164.** The method of claim **147**, further comprising introducing one or more mutations into at least one of the candidate chimeric M2-1 proteins, and identifying at least one mutated candidate chimeric M2-1 protein wherein the altered activity is further altered.
- 165.** The method of claim **164**, further comprising producing at least one recombinant respiratory syncytial virus, the genome or antigenome of which encodes at least one mutated candidate chimeric M2-1 protein.
- 166.** The method of claim **147**, further comprising introducing one or more mutations into at least one RSV M2-1 protein, and identifying at least one candidate mutated RSV M2-1 protein having an altered activity.
- 167.** The method of claim **166**, further comprising producing at least one recombinant respiratory syncytial virus, the genome or antigenome of which encodes at least one candidate mutated RSV M2-1 protein.
- 168.** A recombinant respiratory syncytial virus (RSV) having an attenuated phenotype and comprising an M2-2 protein, which M2-2 protein comprises at least one artificially mutated amino acid residue.

169. The recombinant RSV of claim **168**, wherein the M2-2 protein comprises at least one substituted amino acid residue.

170. The recombinant RSV of claim **168**, wherein the M2-2 protein comprises a deletion of at least one amino acid residue.

171. The recombinant RSV of claim **168**, wherein the M2-2 protein comprises at least one artificially mutated amino acid residue at a position selected from the group consisting of position 1, position 3 and position 7.

172. The recombinant RSV of claim **168**, wherein the M2-2 protein comprises a deletion of amino acid residues 1-2, or wherein the M2-2 protein is an artificial conservative variation of such an M2-2 protein.

173. The recombinant RSV of claim **168**, wherein the M2-2 protein comprises a deletion of amino acid residues 1-6, or wherein the M2-2 protein is an artificial conservative variation of such an M2-2 protein.

174. The recombinant RSV of claim **168**, wherein the M2-2 protein comprises a deletion selected from the group consisting of a deletion of the N-terminal 6 amino acid residues, a deletion of the N-terminal 8 amino acid residues, a deletion of the N-terminal 10 amino acid residues, a deletion of the C-terminal 1 amino acid residue, a deletion of the C-terminal 2 amino acid residues, a deletion of the C-terminal 4 amino acid residues, a deletion of the C-terminal 8 amino acid residues and a deletion of the C-terminal 18 amino acid residues, or wherein the M2-2 protein is an artificial conservative variation of such an M2-2 protein.

175. The recombinant RSV of claim **168**, wherein the M2-2 protein comprises at least one artificially mutated amino acid residue at a position selected from the group consisting of position 2, position 4, position 5, position 6, position 11, position 12, position 15, position 25, position 27, position 34, position 47, position 56, position 58, position 66, position 75, position 80 and position 81.

176. The recombinant RSV of claim **168**, wherein the M2-2 protein comprises at least one amino acid substitution selected from the group consisting of T2A, P4A, K5A, I6A, I6K, D11A, K12A, C15A, R25A, R27A, K34A, H47A, E56A, H58A, D66A, H75A, E80A and

D81A, or wherein the M2-2 protein is an artificial conservative variation of such an M2-2 protein.

177. The recombinant RSV of claim **168**, wherein the recombinant RSV comprises a human RSV of subgroup A, subgroup B or a chimera thereof.

178. A nucleic acid encoding the recombinant RSV of claim **168**.

179. The nucleic acid of claim **178**, wherein the nucleic acid is a DNA.

180. The nucleic acid of claim **179**, wherein the DNA is a cDNA.

181. The nucleic acid of claim **178**, wherein the nucleic acid is an RNA.

182. The nucleic acid of claim **178**, wherein the nucleic acid is an RSV genome or antigenome.

183. A vector comprising the nucleic acid of claim **178**.

184. The vector of claim **183**, comprising a plasmid.

185. The M2-2 protein of claim **168**, or an artificial conservative variation thereof.

186. A nucleic acid encoding the M2-2 protein of claim **185**.

187. A live attenuated respiratory syncytial virus vaccine comprising an immunologically effective amount of the recombinant RSV of claim **168**.

188. The vaccine of claim **187**, further comprising a physiologically acceptable carrier.

189. The vaccine of claim **187**, further comprising an adjuvant.

190. A method for stimulating the immune system of an individual to produce an immune response against RSV, the method comprising administering to the individual the recombinant RSV of claim **168** in a physiologically acceptable carrier.

191. The method of claim **190**, wherein the immune response is a protective immune response.

192. The method of claim **190**, wherein the recombinant RSV is administered to the upper respiratory tract of the individual.

193. The method of claim **192**, wherein the recombinant RSV is administered to the nasopharynx.

194. The method of claim **190**, wherein the recombinant RSV is administered by spray, droplet or aerosol.

195. A method of determining an antibody titer, the method comprising:

contacting a recombinant virus of family Paramyxoviridae, the genome or antigenome of which recombinant virus comprises a marker, and a sample comprising one or more antibodies, in the presence of cells in which the virus can replicate;
permitting replication of the virus; and,
detecting the marker.

196. The method of claim **195**, wherein the recombinant virus comprises a respiratory syncytial virus (RSV).

197. The method of claim **196**, wherein the respiratory syncytial virus comprises a human respiratory syncytial virus of subgroup A, subgroup B or a chimera thereof.

198. The method of claim **197**, wherein the recombinant virus is A-lacZ.

199. The method of claim **197**, wherein the recombinant virus comprises a human RSV of subgroup A in which one or more proteins selected from the group consisting of the G glycoprotein and the F glycoprotein are replaced by one or more homologous proteins of a human RSV of subgroup B.

200. The method of claim **199**, wherein the recombinant virus is B-lacZ.

201. The method of claim **195**, wherein the recombinant virus comprises a metapneumovirus, a sendai virus, a parainfluenza virus, a mumps virus, a newcastle disease virus, a measles virus, a canine distemper virus, or a rinderpest virus.

202. The method of claim **195**, wherein the marker comprises one or more of: a marker nucleic acid that encodes an optically detectable marker protein, a marker nucleic acid that encodes a beta galactosidase protein, a marker nucleic acid that encodes a green fluorescent protein, a marker nucleic acid that encodes a luciferase protein, a marker nucleic acid that encodes a chloramphenicol transferase protein, a marker nucleic acid that encodes a selectable marker protein, a gene that confers cellular resistance to an antibiotic, or a gene conferring resistance to neomycin.

203. The method of claim **195**, wherein the sample comprising one or more antibodies comprises a serum, a bronchial lavage, or a nasal wash.

- 204.** The method of claim **203**, wherein the serum is a peripheral blood-derived serum.
- 205.** The method of claim **195**, wherein contacting a recombinant virus and a sample in the presence of cells comprises combining the virus and the sample and then combining the combined virus and sample with the cells.
- 206.** The method of claim **195**, wherein the virus and the sample are contacted in the presence of one or more complement factors.
- 207.** The method of claim **195**, wherein detecting the marker comprises detecting expression of the marker.
- 208.** The method of claim **207**, wherein detecting expression of the marker comprises quantitating the expression of the marker.
- 209.** The method of claim **207**, further comprising washing and lysing the cells prior to detecting expression of the marker.
- 210.** A composition, comprising:
a recombinant virus of family Paramyxoviridae, the genome or antigenome of which comprises a marker; and,
one or more antibodies.
- 211.** The composition of claim **210**, wherein the recombinant virus comprises a respiratory syncytial virus.
- 212.** The composition of claim **211**, wherein the respiratory syncytial virus comprises a human respiratory syncytial virus of subgroup A, subgroup B or a chimera thereof.
- 213.** The composition of claim **212**, wherein the recombinant virus is A-lacZ.
- 214.** The composition of claim **212**, wherein the recombinant virus comprises a human RSV of subgroup A in which one or more proteins selected from the group consisting of the G glycoprotein and the F glycoprotein are replaced by one or more homologous proteins of a human RSV of subgroup B.
- 215.** The composition of claim **214**, wherein the recombinant virus is B-lacZ.
- 216.** The composition of claim **210**, wherein the recombinant virus comprises a metapneumovirus, a sendai virus, a parainfluenza virus, a mumps virus, a newcastle disease virus, a measles virus, a canine distemper virus, or a rinderpest virus.

217. The composition of claim **210**, wherein the marker comprises one or more of: a marker nucleic acid that encodes an optically detectable marker protein, a marker nucleic acid that encodes a beta galactosidase protein, a marker nucleic acid that encodes a green fluorescent protein, a marker nucleic acid that encodes a luciferase protein, a marker nucleic acid that encodes a chloramphenicol transferase protein, a marker nucleic acid that encodes a selectable marker protein, a gene that confers cellular resistance to an antibiotic, or a gene conferring resistance to neomycin.

218. The composition of claim **210**, further comprising cells in which the virus can replicate.

219. The composition of claim **210**, further comprising one or more complement factors.

220. A recombinant respiratory syncytial virus (RSV) comprising a genome or antigenome comprising a marker, which marker comprises one or more of: a marker nucleic acid that encodes a beta galactosidase protein, a marker nucleic acid that encodes a luciferase protein, a marker nucleic acid that encodes a selectable marker protein, a gene that confers cellular resistance to an antibiotic, or a gene conferring resistance to neomycin.

221. The virus of claim **220**, wherein the recombinant RSV comprises a human RSV of subgroup A, subgroup B or a chimera thereof.

222. The virus of claim **221**, wherein the recombinant virus is A-lacZ.

223. The virus of claim **221**, wherein the recombinant virus comprises a human RSV of subgroup A in which one or more proteins selected from the group consisting of the G glycoprotein and the F glycoprotein are replaced by one or more homologous proteins of a human RSV of subgroup B.

224. The virus of claim **223**, wherein the recombinant virus is B-lacZ.

225. A nucleic acid encoding the recombinant RSV of claim **220**.

226. The nucleic acid of claim **225**, wherein the nucleic acid is a DNA.

227. The nucleic acid of claim **226**, wherein the DNA is a cDNA.

228. The nucleic acid of claim **225**, wherein the nucleic acid is an RNA.

- 229.** The nucleic acid of claim **225**, wherein the nucleic acid is an RSV genome or antigenome.
- 230.** A vector comprising the nucleic acid of claim **225**.
- 231.** The vector of claim **230**, comprising a plasmid.
- 232.** A recombinant virus of family Paramyxoviridae, which recombinant virus comprises a genome or antigenome comprising a marker, and which recombinant virus comprises a metapneumovirus, a sendai virus, a parainfluenza virus, a mumps virus, or a canine distemper virus.
- 233.** The virus of claim **232**, wherein the marker comprises one or more of: a nucleic acid that encodes an optically detectable marker protein, a marker nucleic acid that encodes a beta galactosidase protein, a marker nucleic acid that encodes a green fluorescent protein, a marker nucleic acid that encodes a luciferase protein, a marker nucleic acid that encodes a chloramphenicol transferase protein, a marker nucleic acid that encodes a selectable marker protein, a gene that confers cellular resistance to an antibiotic, or a gene conferring resistance to neomycin.
- 234.** A nucleic acid encoding the virus of claim **232**.
- 235.** The nucleic acid of claim **234**, wherein the nucleic acid is a DNA.
- 236.** The nucleic acid of claim **235**, wherein the DNA is a cDNA.
- 237.** The nucleic acid of claim **234**, wherein the nucleic acid is an RNA.
- 238.** The nucleic acid of claim **234**, wherein the nucleic acid is a viral genome or antigenome.
- 239.** A vector comprising the nucleic acid of claim **234**.
- 240.** The vector of claim **239**, comprising a plasmid.